AZABICYCLO DERIVATIVES AS MUSCARINIC RECEPTOR ANTAGONISTS

Field of the Invention

This invention generally relates to muscarinic receptor antagonists which are useful, among other uses, for the treatment of various diseases of the respiratory, urinary and gastrointestinal systems mediated through muscarinic receptors. Specifically, the invention relates to derivatives of azabicyclo compounds, including, for example, 6-substituted azabicyclo[3.1.0] hexanes, as well as pharmaceutical compositions containing such compounds and methods of treating diseases mediated through muscarinic receptors.

Background of the Invention

Muscarinic receptors as members of the G Protein Coupled Receptors (GPCRs) are composed of a family of 5 receptor sub-types (M_1, M_2, M_3, M_4 and M_5) and are activated by the neurotransmitter acetylcholine. These receptors are widely distributed on multiple organs and tissues and are critical to the maintenance of central and peripheral cholinergic neurotransmission. The regional distribution of these receptor sub-types in the brain and other organs has been documented. For example, the M_1 subtype is located primarily in neuronal tissues such as cereberal cortex and autonomic ganglia, the M_2 subtype is present mainly in the heart where it mediates cholinergically induced bradycardia, and the M_3 subtype is located predominantly on smooth muscle and salivary glands (*Nature*, 323, p.411 (1986); *Science*, 237, p.527 (1987)).

A review in *Current Opinions in Chemical Biology*, 3, p. 426 (1999), as well as in *Trends in Pharmacological Sciences*, 22, p. 409 (2001) by Eglen et. al., describes the biological potentials of modulating muscarinic receptor subtypes by ligands in different disease conditions, such as Alzheimer's Disease, pain, urinary disease condition, chronic obstructive pulmonary disease, and the like.

A review in *J. Med. Chem.*, 43, p. 4333 (2000), by Felder et. al. describes therapeutic opportunities for muscarinic receptors in the central nervous system and elaborates on muscarinic receptor structure and function, pharmacology and their therapeutic uses.

The pharmacological and medical aspects of the muscarinic class of acetylcholine agonists and antagonists are presented in a review in *Molecules*, 6, p. 142 (2001).

Birdsall et. al. in *Trends in Pharmacological Sciences*, 22, p. 215 (2001) have also summarized the recent developments on the role of different muscarinic receptor subtypes using different muscarinic receptor of knock out mice.

Muscarinic agonists such as muscarine and pilocarpine and antagonists such as atropine have been known for over a century, but little progress has been made in the discovery of receptor subtype-selective compounds, making it difficult to assign specific functions to the individual receptors. Although classical muscarinic antagonists such as atropine are potent bronchodilators, their clinical utility is limited due to high incidence of both peripheral and central adverse effects such as tachycardia, blurred vision, dryness of mouth, constipation, dementia, etc. Subsequent development of the quarterly derivatives of atropine such as ipratropium bromide are better tolerated than parenterally administered options, but most of these are not ideal anti-cholinergic bronchodilators, due to lack of selectivity for muscarinic receptor sub-types, resulting in dose-limiting side-effects such as thirst, nausea, mydriasis and those associated with the heart such as tachycardia mediated by the M_2 receptor.

Annual Review of Pharmacological Toxicol., 41, p. 691 (2001), describes the pharmacology of the lower urinary tract infections. Although anti-muscarinic agents such as oxybutynin and tolterodine that act non-selectively on muscarinic receptors have been used for many years to treat bladder hyperactivity, the clinical effectiveness of these agents has been limited due to the side effects such as dry mouth, blurred vision and constipation. Tolterodine is considered to be generally better tolerated than oxybutynin. (Steers et. al., in *Curr. Opin. Invest. Drugs*, 2, 268; Chapple et. al., in *Urology*, 55, 33; Steers et al., Adult and Pediatric Urology, ed. Gillenwatteret al., pp 1220-1325, St. Louis, MO; Mosby. 3rd edition (1996)).

There remains a need for development of new highly selective muscarinic antagonists which can interact with distinct subtypes, thus avoiding the occurrence of adverse effects.

Compounds having antagonistic activity against muscarinic receptors have been described in Japanese patent application Laid Open Number 92921/1994 and 135958/1994; WO 93/16048; U.S. Patent No. 3,176,019; GB 940,540; EP 0325 571; WO 98/29402; EP 0801067; EP 0388054; WO 9109013; U.S. Patent No. 5,281,601. Also, U.S. Patent Nos. 6,174,900, 6,130,232 and 5,948,792; WO 97/45414 are related to

1,4-disubstituted piperidine derivatives; WO 98/05641 describes fluorinated, 1,4-disubstitued piperidine derivatives; WO 93/16018 and WO96/33973 are other references of interest. US Patent No. 5,397,800 discloses 1-azabicyclo[2.2.1]heptanes. US Patent No.5, 001,160 describes 1-aryl-1-hydroxy-1-substituted-3-(4-substituted-1-piperazinyl)-2-propanones. WO 01/42213 describes 2-biphenyl-4-piperidinyl ureas. WO 01/42212 describes carbamate derivatives. WO 01/90081 describes amino alkyl lactam. WO 02/53564 describes novel quinuclidine derivatives. WO 02/00652 describes carbamates derived from arylalkyl amines. WO 02/06241 describes 1,2,3,5-tetrahydrobenzo(c)azepin-4-one derivatives.

A report in *J. Med. Chem.*, 44, p. 984 (2002), describes cyclohexylmethyl piperidinyl triphenylpropioamide derivatives as selective M_3 antagonist discriminating against the other receptor subtypes.

Summary of the Invention

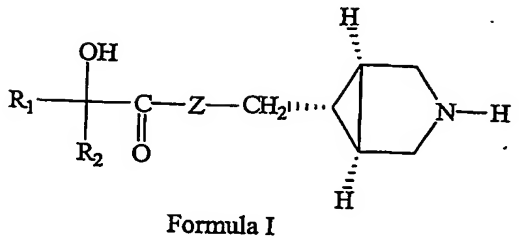
In one aspect, azabicyclo derivatives, including, for example, 6-substituted azabicyclo[3.1.0]hexanes, 2,6- and 4,6-disubstituted derivatives and 2,4,6-trisubstituted derivatives are provided as muscarinic receptor antagonists which can be useful as safe and effective therapeutic or prophylactic agents for the treatment of various diseases of the respiratory, urinary and gastrointestinal systems. Also provided are processes for synthesizing such compounds.

In another aspect, pharmaceutical compositions containing such compounds are provided together with acceptable carriers, excipients or diluents which can be useful for the treatment of various diseases of the respiratory, urinary and gastrointestinal systems.

The enantiomers, diastereomers, N-oxides, polymorphs, pharmaceutically acceptable salts and pharmaceutically acceptable solvates of these compounds as well as metabolites having the same type of activity are also provided, as well as pharmaceutical compositions comprising the compounds, their metabolites, enantiomers, diastereomers, N-oxides, polymorphs, solvates or pharmaceutically acceptable salts thereof, in combination with a pharmaceutically acceptable carrier and optionally included excipients.

Other aspects will be set forth in the description which follows, and in part will be apparent from the description or may be learnt by the practice of the invention.

In accordance with one aspect, there are provided compounds having the structure of Formula I:



Formula I

and their pharmaceutically acceptable salts, pharmaceutically acceptable solvates, esters, enantiomers, diastereomers, N-oxides, polymorphs, metabolites, wherein

R_1 and R_2 are independently selected from C_1-C_6 alkyl, C_3-C_7 cycloalkyl or optionally substituted phenyl wherein optional substituent(s) can be selected from C_1-C_3 alkyl, C_1-C_3 alkoxy or halogen;

Z can represent oxygen or NR_3 wherein R_3 represents hydrogen or C_1-C_3 alkyl.

In accordance with a second aspect, there is provided a method for treatment or prophylaxis of an animal or a human suffering from a disease or disorder of the respiratory, urinary and gastrointestinal systems, wherein the disease or disorder is mediated through muscarinic receptors. The method includes administration of at least one compound having the structure of Formula I.

In accordance with a third aspect, there is provided a method for treatment or prophylaxis of an animal or a human suffering from a disease or disorder associated with muscarinic receptors, comprising administering to a patient in need thereof, an effective amount of a muscarinic receptor antagonist compound as described above.

In accordance with a fourth aspect, there is provided a method for treatment or prophylaxis of an animal or a human suffering from a disease or disorder of the respiratory system such as bronchial asthma, chronic obstructive pulmonary disorders (COPD), pulmonary fibrosis, and the like; urinary system which induce such urinary disorders as urinary incontinence, lower urinary tract symptoms (LUTS), etc.; and gastrointestinal system such as irritable bowel syndrome, obesity, diabetes and gastrointestinal hyperkinesis with compounds as described above, wherein the disease or disorder is associated with muscarinic receptors.

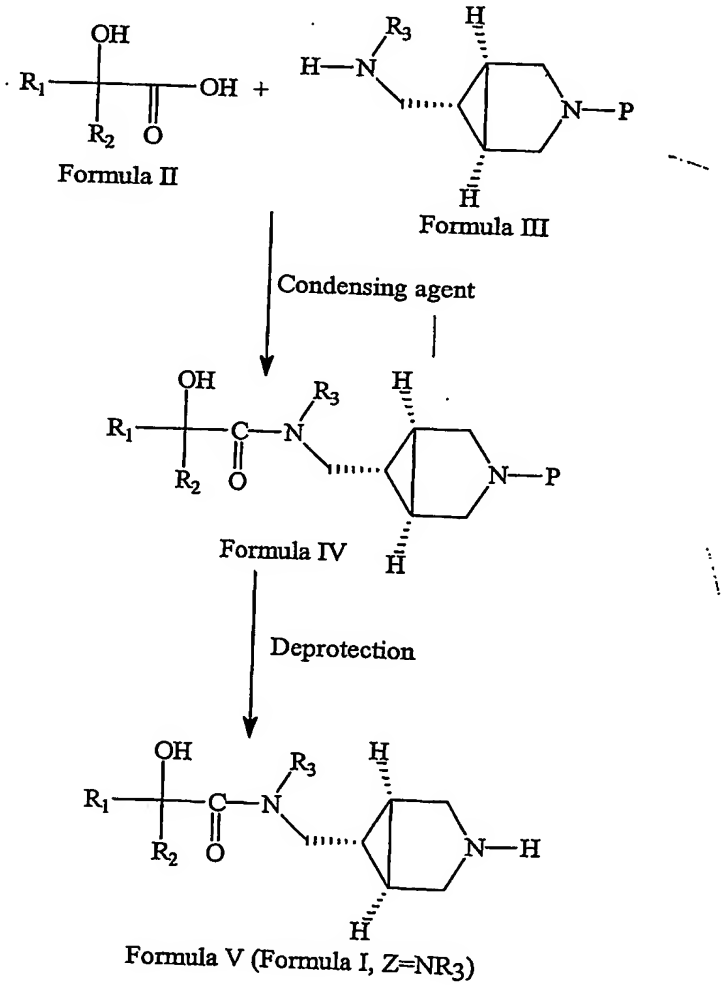
In accordance with a fifth aspect, there are provided processes for preparing the compounds as described above.

The compounds described herein exhibit significant potency in terms of their activity, as determined by *in vitro* receptor binding and functional assays and *in vivo* experiments using anaesthetized rabbits. The compounds that were found active *in vitro* were tested *in vivo*. Some of the compounds are potent muscarinic receptor antagonists with high affinity towards M_3 receptors. Therefore, pharmaceutical compositions for the possible treatment for the disease or disorders associated with muscarinic receptors are provided. In addition, the compounds can be administered orally or parenterally.

Detailed Description of the Invention

The compounds presented herein may be prepared by methods represented by the following reaction sequences as shown in Schemes I and II:

Scheme I



The compounds of Formula V may be prepared, for example, by the reaction sequence as shown in Scheme I. The preparation comprises reacting a compound of Formula II with a compound of Formula III, wherein

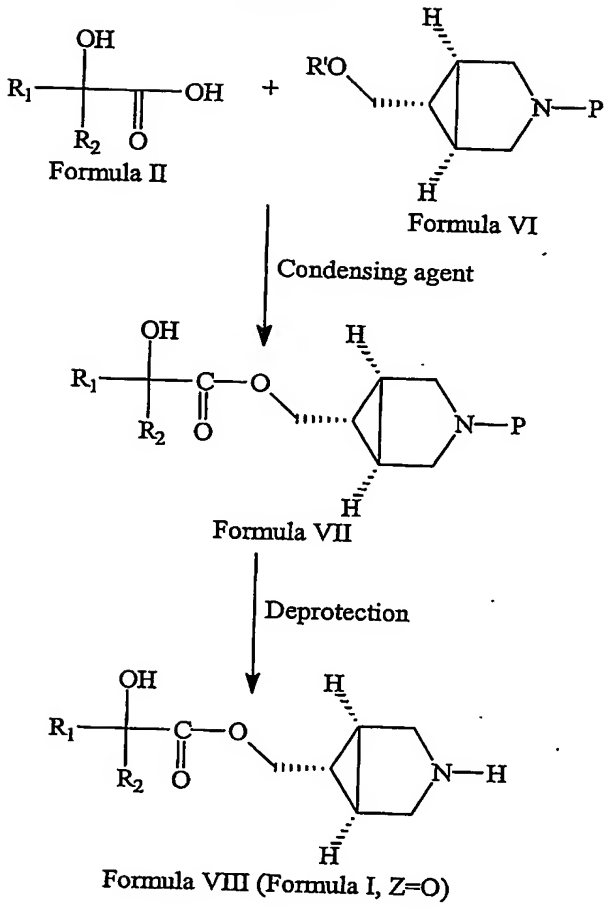
R_1 and R_2 are independently selected from C_1-C_6 alkyl, C_3-C_7 cycloalkyl or optionally substituted phenyl wherein optional substituent(s) is/are selected from C_1-C_3 alkyl, C_1-C_3 alkoxy or halogen;

R_3 represents hydrogen or C_1-C_3 alkyl and

P is any protecting group for an amino group, for example, benzyl or t-butyloxy carbonyl groups.

The reaction between a compound of Formula II and a compound of Formula III can take place in the presence of N-methylmorpholine and 1-hydroxybenzotriazole and a condensing agent (for example, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC), 1,3-dicyclohexylcarbodiimide (DCC) or 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU)), in a solvent (such as N,N-dimethylformamide, dimethylsulfoxide, toluene, xylene or chloroform, at temperatures ranging from about 0 to about 140°C), to give a protected compound of Formula IV which on deprotection in the presence of a deprotecting agent (for example, palladium on carbon and hydrogen, ammonium formate and palladium on carbon, trifluoroacetic acid (TFA) or hydrochloric acid) in an organic solvent (for example, methanol, ethanol, tetrahydrofuran or acetonitrile, at temperatures ranging from about 10 to about 50°C) gives an unprotected compound of Formula V.

Scheme II



The compounds of Formula VIII may be prepared, for example, by the reaction sequence as shown in Scheme II. The preparation comprises reacting a compound of Formula II with a compound of Formula VI, wherein

R_1 and R_2 are independently selected from C_1-C_6 alkyl, C_3-C_7 cycloalkyl or optionally substituted phenyl wherein optional substituent(s) is/are selected from C_1-C_3 alkyl, C_1-C_3 alkoxy or halogen;

R′ is any protecting group for hydroxy group, for example, p-toluene sulfonyl or methane sulfonyl and

P is any protecting group for an amino group, for example, benzyl or t-butyloxy carbonyl groups.

The reaction between a compound of Formula II and a compound of Formula VI can take place in the presence of a condensing agent (for example, 1,8-diazabicyclo[5.4.0]undecan-7-ene (DBU) or 1,4-diazabicyclo[2.2.2]octane (DABCO), in a

solvent (such as benzene, toluene or xylene, at temperatures ranging from about 0 to about 140°C), to give a protected compound of Formula VII which on deprotection in the presence of a deprotecting agent (for example, palladium on carbon and hydrogen or ammonium formate and palladium on carbon) in an organic solvent (for example, methanol or ethanol, at temperatures ranging from about 10 to about 50°C) gives an unprotected compound of Formula VIII.

In the above scheme, where specific bases, condensing agents, protecting groups, deprotecting agents, solvents, catalysts, temperatures, etc. are mentioned, it is to be understood that other bases, condensing agents, protecting groups, deprotecting agents, solvents, catalysts, temperatures, etc. known to those skilled in the art may be used. Similarly, the reaction temperature and duration may be adjusted according to the desired needs.

Suitable salts of the compounds represented by the Formula I were prepared so as to solubilize the compound in aqueous medium for biological evaluations, as well as to be compatible with various dosage formulations and also to aid in the bioavailability of the compounds. Examples of such salts include pharmacologically acceptable salts such as inorganic acid salts (for example, hydrochloride, hydrobromide, sulphate, nitrate and phosphate), organic acid salts (for example, acetate, tartarate, citrate, fumarate, maleate, tolounesulphonate and methanesulphonate). When carboxyl groups are included in the Formula I as substituents, they may be present in the form of an alkaline or alkali metal salt (for example, sodium, potassium, calcium, magnesium, and the like). These salts may be prepared by various techniques, such as treating the compound with an equivalent amount of inorganic or organic, acid or base in a suitable solvent.

Particular compounds are shown here:

N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 1);

N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide tartarate salt (Compound No. 2);

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide (Compound No. 3);

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide hydrochloride salt (Compound No. 4);

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(3-pentyl)-2-hydroxy-2-phenylacetamide (Compound No. 5);

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 6);

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 7);

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-(N-methyl) phenylacetamide hydrochloride salt (Compound No. 8);

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-methyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 9);

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 10);

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(3-pentyl)-2-hydroxy-2-phenylacetic acid ester (Compound No. 11);

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-methyl-2-hydroxy-2-phenylacetamide (Compound No. 12);

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 13);

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(m-methylphenyl)-2-hydroxy-2-phenylacetic acid ester (Compound No. 14);

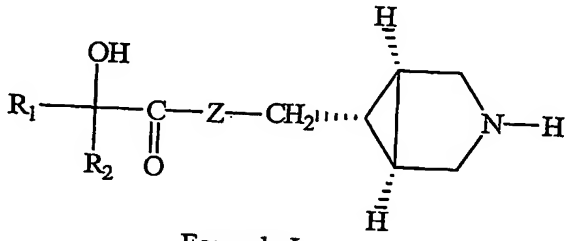
(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-fluorophenyl)-2-hydroxy-2-phenylacetamide (Compound No. 15);

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-methylphenyl)-2-hydroxy-2-phenylacetamide (Compound No. 16);

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-fluorophenyl)-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 17);

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-methylphenyl)-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 18).

Table 1



Formula I

Compound No.	R_1	R_2	Z
1	$-C_6H_5$	$-C_6H_5$	$N-CH_3$
2 (tartarate salt)	$-C_6H_5$	$-C_6H_5$	$N-CH_3$
3 (2R, 2S)	$-C_6H_5$	Isopropyl	NH-
4 (HCl salt) (2R, 2S)	$-C_6H_5$	Isopropyl	NH-
5 (2R, 2S)	$-C_6H_5$	3-pentyl	NH-
6 (2R, 2S)	$-C_6H_5$	Cyclopentyl	O
7 (2R, 2S)	$-C_6H_5$	Cyclopentyl	$-N-CH_3$
8 (HCl salt) (2R, 2S)	$-C_6H_5$	Cyclopentyl	$-N-CH_3$
9 (2R, 2S)	$-C_6H_5$	$-CH_3$	O
10 (2R, 2S)	$-C_6H_5$	Isopropyl	O
11 (2R, 2S)	$-C_6H_5$	3-pentyl	O
12 (2R, 2S)	$-C_6H_5$	$-CH_3$	NH-
13 (2R)	$-C_6H_5$	Isopropyl	$-N-CH_3$
14 (2R, 2S)	$-C_6H_5$	$m-CH_3-C_6H_4$	O
15 (2R, 2S)	$-C_6H_5$	$p-F-C_6H_4$	NH-

16 (2R, 2S)	$-C_6H_5$	$p-CH_3-C_6H_4$	NH-
17 (2R)	$-C_6H_5$	$p-F-C_6H_4$	$-N-CH_3$
18 (2R)	$-C_6H_5$	$p-CH_3-C_6H_4$	$-N-CH_3$

Because of their valuable pharmacological properties, the compounds described herein may be administered to an animal for treatment orally, or by a parenteral route. The pharmaceutical compositions described herein can be produced and administered in dosage units, each unit containing a certain amount of at least one compound described herein and/or at least one physiologically acceptable addition salt thereof. The dosage may be varied over extremely wide limits as the compounds are effective at low dosage levels and relatively free of toxicity. The compounds may be administered in the low micromolar concentration, which is therapeutically effective, and the dosage may be increased as desired up to the maximum dosage tolerated by the patient.

The compounds described herein can be produced and formulated as their enantiomers, diastereomers, N-Oxides, polymorphs, solvates and pharmaceutically acceptable salts, as well as metabolites having the same type of activity. Pharmaceutical compositions comprising the molecules of Formula I or metabolites, enantiomers, diastereomers, N-oxides, polymorphs, solvates or pharmaceutically acceptable salts thereof, in combination with pharmaceutically acceptable carrier and optionally included excipient can also be produced.

The examples mentioned below demonstrate general synthetic procedures, as well as specific preparations of particular compounds. The examples are provided to illustrate the details of the invention and should not be constrained to limit the scope of the present invention.

Examples

Various solvents, such as acetone, methanol, pyridine, ether, tetrahydrofuran, hexanes, and dichloromethane, were dried using various drying reagents according to procedures described in the literature. IR spectra were recorded as nujol mulls or a thin neat film on a Perkin Elmer Paragon instrument, Nuclear Magnetic Resonance (NMR) were recorded on a Varian XL-300 MHz instrument using tetramethylsilane as an internal standard.

Example 1: Preparation of N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 1)

Step a: Synthesis of methane sulfonic acid 3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl ester

To a solution of (3-benzyl-3-azabicyclo[3.1.0]hex-6-yl) methanol (prepared following *Synlett*, 1996; 1097) (5.2g, 25.6 mmole) in dichloromethane at 0°C triethylamine (10.6 mL, 76.8 mmole) and methane sulphonyl chloride (4 mL, 51.2 mmole) was added. It was gradually warmed to an ambient temperature and stirred for overnight. It was quenched by addition of saturated aqueous sodium bicarbonate solution and organic layer was separated to give solution of crude product. This was washed with water, brine and dried over anhydrous sodium sulphate and the evaporated to give crude product. The crude product was purified by column chromatography using silica gel with hexane-triethylamine (99.1) as eluant to give the required product as pale yellow clear liquid (2.2 g, 30%).

Step b: Synthesis of (3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl) methylamine

To a solution of methane sulfonic acid 3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl ester

(2.4 g, 8.5 mmol) in methanol (20 ml) in a steel bomb, aqueous 40% methylamine solution (25 ml) was added. The steel bomb was tightened and warmed to 85-90°C for about 15 hour. It was cooled down to an ambient temperature and then to -78°C and was opened up. The mixture was transferred to a round bottom flask and solvent was evaporated, diluted with water, dilulte hydrochloric acid and extracted with ethyl acetate. Organic layer was separated and discarded. The aqueous layer was basified with 10% aqueous sodium hydroxide solution to pH 12-13. It was extracted with dichloromethane and dried over anhydrous sodium sulphate. The filtered dichloromethane layer was evaporated to give the required compound as yellow liquid (1.8 g, 98%).

Step c: Synthesis of N-[(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide

To a cold solution of benzillic acid (1.9 g, 8.33 mmol, commercially available) and (3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl)methylamine (1.8 g, 8.33 mmol) in dimethyformamide (20 ml) at 0°C, N-methylmorpholine (1.8 ml, 16.6 mmol) and 1-

hydroxy benzotriazole (1.12 g, 8.33 mmol) were added and the mixture was stirred for about 45 min. To it 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.6 g, 8.33 mmol) was added and the mixture was gradually warmed to an ambient temperature and stirred for overnight. It was quenched by addition of water and compound was extracted with ethyl acetate. The organic layer was separated and washed with water, brine and dried over anhydrous sodium sulphate. The organic layer was filtered and evaporated to give crude product. The crude product was purified by silica gel column chromatography using hexane-ethyl acetate (4:1 to 2:1) as eluant to give the required product as colourless sticky solid (1.3 g, 36%)

Step d: Synthesis of N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide

To a solution of N-[(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide (1.3g, 3.05mmole) in methanol (20 mL), catalyst palladium on carbon (10%, wet) was added and a 3-way hydrogenation tap fixed with filled hydrogen ballon was fixed over it. The air was evacuated and purged with hydrogen. It was stirred for about 5 hours at an ambient temperature. The catalyst was filtered off over celite and washed with methanol. Filterate was evaporated to give the required product as colourless sticky liquid (0.95 g, 93%).

The compound exhibited a melting point of 72.4-73.7 ^{0}C. Infrared spectral data showed (DCM): 1627.9 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 8.42-8.29 (m, 10H), 4.52 (s, 2H), 4.17 (s, 2H), 3.94-4.00 (m, 3H), 3.58-3.64 (m, 4H), 2.45-2.58 (m, 2H), 1.91 (m, 1H). The mass spectrum showed peaks at m/e of: 337 (M+1).

Example 2: Preparation of N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide tartarate salt (Compound No. 2)

To a solution of N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-phenyl-2-hydroxy-2-(N-methyl) phenylacetamide (0.933, 2.77mmole, prepared in Example 1, step d)) in ethanol (25 mL), L(+) tartaric acid (416 mg, 2.77mmole) was added and the solution was stirred for 1 hour at room temperature. A white precipitate appeared. It was heated to 50-55 ^{0}C for 30 minutes and solvent was evaporated to half amount. Dry ether

was added to it and white precipitate was filtered off and washed with plenty of ether. The dry white powder was attained (1.3g, 96%).

The compound exhibited a melting point of 101-103 ^{0}C.

Similarly, the following compounds were prepared following the procedure described in Example 1.

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 13)

Infrared spectral data showed (DCM): 1619.7 cm^{-1}. ^{1}HNMR spectral data showed (D_2O): δ 7.25-7.45 (m, 5H), 3.45-3.51 (m, 1H), 2.80-2.83 (m, 6H), 1.94-1.96 (brs, 3H), 1.24-1.33 (m, 3H), 0.86-0.98 (m, 6H). The mass spectrum showed peaks at m/e of: 303 (M+H).

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-fluorophenyl)-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 17)

^{1}HNMR spectral data showed ($CDCl_3$): δ 7.36-7.04 (m, 9H), 3.49-3.43 (m, 2H), 3.08-2.60 (m, 8H), 1.40-1.36 (m, 2H), 1.24-1.33 (m, 3H), 0.86-0.98 (m, 6H). The mass spectrum showed peaks at m/e of: 303 (M+H).

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-methylphenyl)-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 18)

^{1}HNMR spectral data showed (DMSO): δ 7.46-7.33 (m, 5H), 4.59 (s, 2H), 3.54-3.46 (m, 10H), 3.17-3.05 (m, 3H), 1.36-1.28 (m, 2H).

(2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-(N-methyl) phenylacetamide (Compound No. 7)

Example 3: (2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-(N-methyl) phenylacetamide hydrochloride salt (Compound No. 8)

To a solution of (2R)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-(N-methyl) phenylacetamide in dichloromethane (14.0 mL), etahnolic hydrochloride (3.5 N, 2.1 mL) was added at 0-5 ^{0}C and stirred for about 30 minutes at 20-25 ^{0}C. The solvent was removed under reduced pressure and the residue was

triturated with n-hexane to get a solid. The solid so obtained was filtered and washed with hexane and dried ubder vacuum to get the dried product in 90.1 yield.

Infrared spectral data showed (DCM): 1617.6 cm^{-1}. ^{1}HNMR spectral data showed (D_2O) : δ 7.45-7.52 (m, 5H), 3.42-3.50 (m, 4H), 3.22-3.29 (m, 2H), 2.90 (s, 3H), 1.80 (m, 1H), 1.40-1.50 (m, 8H), 1.22-1.27 (m, 2H), 1.10 (m, 1H). The mass spectrum showed peaks at m/e of: 329 (M+H).

Example 4: Preparation of (2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide (Compound No. 3)

Step a: Synthesis of (3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl) amine

This compound was synthesized following the procedure described in EP 0413 455.

Step b: Synthesis of N-[(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide

To a cold solution of 2-isopropyl-2-hydroxy-2-phenylacetic (prepared following *J. Amer. Chem. Soc.*, 1953; 75: 2654 and *J. Org. Chem.*, 2000; 65:6283) (1.9 g, 8.33 mmol,) and (3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl)amine (prepared following the procedure described in EP 0413455) (1.8 g, 8.33 mmol) in dimethyformamide (20 ml) at 0°C, N-methylmorpholine (1.8 ml, 16.6 mmol) and 1-hydroxy benzotriazole (1.12 g, 8.33 mmol) were added and the mixture was stirred for about 45 min. To it 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.6 g, 8.33 mmol) was added and the mixture was gradually warmed to an ambient temperature and stirred for overnight. It was quenched by addition of water and compound was extracted with ethyl acetate. The organic layer was separated and washed with water, brine and dried over anhydrous sodium sulphate. The organic layer was filtered and evaporated to give crude product. The crude product was purified by silica gel column chromatography using hexane-ethyl acetate (4:1 2:1) as eluant.

Step c: Synthesis of (2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide

To a solution of N-[(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide (1.3g, 30.5mmole) in dry methanol (25.0 mL), 5% palladium on carbon (0.2 g), (50% wet) was added under nitrogen. Then anhydrous ammonium formate (0.8 g, 12.38 mmole) was added under stirring and the reaction mixture was refluxed for half an hour under nitrogen atmosphere. Cooled to room temperature and the reaction mixture was filtered through a bed of hyflo. The hyflo bed was washed with methanol (75.0 mL), ethyl acetate (25.0 mL) and water (25.0 mL). The filterate was concentrated under vaccum. The residue was diluted with water and pH of the resulting solution was adjusted to (pH~14) with 1N NaOH. Extracted with ethyl acetate (2x50 mL) and the ethyl acetate layer was washed with water and brine solution. Dried over anhydrous sodium sulphate and concentrated to give the title compound.

Infrared spectral data showed (DCM): 1654 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.60-7.62 (m, 2H), 7.24-7.37 (m, 3H), 6.74 (s, 1H), 3.06-3.16 (m, 2H), 2.79-2.94 (m, 5H), 1.26-1.31 (m, 2H), 1.00 (d, J=6Hz, 3H), 0.72-0.77 (m, 4H). The mass spectrum showed peaks at m/e of: 289 (M+1).

Example 5: Preparation of (2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide hydrochloride salt (Compound No. 4)

To a solution of (2R or 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetamide (1.4 g, 4.9 mmole) in dichloromethane (14.0 mL), etahnolic hydrochloride (3.5 N, 2.1 mL, 7.3 mmole) was added at 0-5 °C and stirred for about 30 minutes at 20-25 °C. The solvent was removed under reduced pressure and the residue was triturated with n-hexane to get a solid. The solid so obtained was filtered and washed with hexane and dried ubder vacuum to get the dried product in 95.1 (1.5 g) yield.

The compound exhibited a melting point of 70 °C (softening start). Infrared spectral data showed (DCM): 1641.1 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.63-7.65 (m, 2H), 7.40-7.47 (m, 3H), 3.30-3.37 (m, 4H), 3.14-3.16 (m, 2H), 2.90-2.93 (m, 1H), 1.74 (s, 2H), 1.21-1.23 (m, 1H), 1.00-1.01 (m, 3H), 0.81-0.83 (m, 3H).

Similarly, the following compounds were prepared following the procedure described in Example 4.

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(3-pentyl)-2-hydroxy-2-phenylacetamide (Compound No. 5)

Infrared spectral data showed (DCM): 1651.7 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.61-7.64 (m, 2H), 7.27-7.35 (m, 3H), 6.83 (s, 1H), 2.83-3.16 (m, 7H), 2.35 (m, 2H), 1.90-2.00 (m, 1H), 0.78-1.47 (m, 14H). The mass spectrum showed peaks at m/e of: 317 (M+1)

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-methyl-2-hydroxy-2-phenylacetamide (Compound No. 12)

Infrared spectral data showed (DCM): 1655.5 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.54-7.56 (m, 2H), 7.28-7.37 (m, 3H), 6.76 (brs, 1H), 3.05-3.20 (m, 2H), 2.80-2.93 (m, 4H), 1.79 (s, 3H), 1.22-1.32 (m, 2H), 0.76-0.80 (m, 1H). The mass spectrum showed peaks at m/e of: 261(M+1).

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-fluorophenyl)-2-hydroxy-2-phenylacetamide (Compound No. 15)

^{1}HNMR spectral data showed ($CDCl_3$): δ 7.45-7.03 (m, 9H), 6.70 (brs, 1H), 3.26-3.22 (m, 2H), 2.96-2.83 (m, 4H), 1.34-1.30 (m, 3H). The mass spectrum showed peaks at m/e of: 341.39 (M+1)

(2R, 2S)-N-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(p-methylphenyl)-2-hydroxy-2-phenylacetamide (Compound No. 16)

^{1}HNMR spectral data showed ($CDCl_3$): δ 7.44-7.14 (m, 9H), 6.70 (brs, 1H), 3.25-3.21 (m, 2H), 2.97-2.84 (m, 4H), 2.39-2.29 (m, 3H), 1.30-1.28 (m, 3H). The mass spectrum showed peaks at m/e of: 337.40 (M+1).

Example 6: Preparation of (2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 6)

Step a: Synthesis of [(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-phenylacetic acid ester

To a cold solution of 2-cyclopentyl-2-hydroxy-2-phenylacetic acid (1.9 g, 8.33 mmol) (prepared following *J. Amer. Chem. Soc.*, 1953; 75: 2654 and *J. Org. Chem.*, 2000; 65:6283) and methane sulfonic acid 3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl ester

(prepared in Example 1, step a) (2.4 g, 8.5 mmol) in dimethyformamide (20 ml) at 0°C, 1,8-diazabicyclo[5.4.0]undecan-7-ene (DBU) (1.6 g, 8.33 mmol) was added and the mixture was gradually warmed to an ambient temperature and stirred for overnight. It was quenched by addition of water and compound was extracted with ethyl acetate. The organic layer was separated and washed with water, brine and dried over anhydrous sodium sulphate. The organic layer was filtered and evaporated to give crude product. The crude product was purified by silica gel column chromatography.

Step b: Synthesis of [(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-cyclopentyl-2-hydroxy-2-phenylacetic acid ester

To a solution of [(1α, 5α, 6α)-3-benzyl-3-azabicyclo[3.1.0]hex-6-yl-methyl]2-cyclopentyl-2-hydroxy-2-phenylacetic acid ester in dry methanol (25.0 mL), 5% palladium on carbon (0.2 g), (50% wet) was added under nitrogen. Then anhydrous ammonium formate (0.8 g, 12.38 mmole) was added under stirring and the reaction mixture was refluxed for half an hour under nitrogen atmosphere. Cooled to room temperature and the reaction mixture was filtered through a bed of hyflo. The hyflo bed was washed with methanol (75.0 mL), ethtl acetate (25.0 mL) and water (25.0 mL). The filterate was concentrated under vaccum. The residue was diluted with water and pH of the resulting solution was adjusted to (pH~14) with 1N NaOH. Extracted with ethyl acetate (2x50 mL) and the ethyl acetate layer was washed with water and brine solution. Dried over anhydrous sodium sulphate and concentrated to give the title compound.

^{1}HNMR spectral data showed ($CDCl_3$): δ 7.67-7.64 (m, 2H), 7.36-7.28 (m, 3H), 4.13-4.05 (m, 2H), 2.97-2.86 (m, 4H), 2.29-1.50 (m, 12H). The mass spectrum showed peaks at m/e of: 316.31 (M+1).

Similarly, the following compounds were prepared following the procedure described in Example 6.

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-methyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 9)

Infrared spectral data showed (DCM): 1729.7 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.55-7.58 (m, 2H), 7.29-7.38 (m, 3H), 4.02-4.12 (m, 2H), 2.82-2.94 (m, 4H), 1.71 (s, 3H), 1.48 (s, 2H), 0.93-0.97 (m, 1H). The mass spectrum showed peaks at m/e of: 262 (M+1).

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-isopropyl-2-hydroxy-2-phenylacetic acid ester (Compound No. 10)

Infrared spectral data showed (DCM): 1723.8 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.65-7.67 (m, 2H), 7.24-7.37 (m, 3H), 4.05-4.16 (m, 2H), 2.81-2.93 (m, 4H), 2.61-2.66 (m, 1H), 1.29-1.39 (m, 3H), 0.94-1.02 (m, 3H), 0.71 (d, J=6Hz, 2H). The mass spectrum showed peaks at m/e of: 290 (M+1)

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(3-pentyl)-2-hydroxy-2-phenylacetic acid ester (Compound No. 11)

Infrared spectral data showed (DCM): 1721.4 cm^{-1}. ^{1}HNMR spectral data showed ($CDCl_3$): δ 7.64-7.67 (m, 2H), 7.29-7.37 (m, 3H), 4.02-4.11 (m, 2H), 2.92-3.02 (m, 4H), 2.15-2.19 (m, 1H), 1.42-1.51 (m, 4H), 1.09-1.29 (m, 3H), 0.98-1.03 (m, 3H), 0.71-0.76 (m, 3H). The mass spectrum showed peaks at m/e of: 318 (M+1).

(2R, 2S)-[(1α, 5α, 6α)-3-azabicyclo[3.1.0]hex-6-yl-methyl]-2-(m-methylphenyl)-2-hydroxy-2-phenylacetic acid ester (Compound No. 14)

^{1}HNMR spectral data showed ($CDCl_3$): δ 7.43-7.12 (m, 14H), 4.18-4.16 (m, 2H), 3.03-2.91 (m, 4H), 2.33-2.28 (m, 3H), 1.30-1.28 (m, 3H). The mass spectrum showed peaks at m/e of: 338.34 (M+1).

Biological Activity

Radioligand Binding Assays: The affinity of test compounds for M_2 and M_3 muscarinic receptor subtypes was determined by [^{3}H]-N-methylscopolamine binding studies, using rat heart and submandibular gland, respectively, as described by Moriya et al., (Life Sci., 1999; 64(25):2351-2358) with minor modifications as follows. The membrane preparation was done with the following modifications: a low spin step of 500g for 10 minutes at 4°C was used; the buffer was 20 mM HEPES, 10 mM EDTA, at pH 7.4; the high speed spin was done at 40,000g and the homogenate was passed through a filter gauge before any spinning. The assay conditions were modified as follows: the assay volume was 250 μL; the incubation time was 3 hours; the PE concentration was 0.1%; the filtermat used was GF/B from Wallac; the scintillant used was Supermix from Wallac; the amount of scintillant was 500 μL/well; and the counter used was a 1450 microbeta PLUS, from Wallac.

Membrane preparation: Submandibular glands and heart were isolated and placed in ice cold homogenising buffer (HEPES 20mM, 10mM EDTA, pH 7.4) immediately after sacrifice. The tissues were homogenised in 10 volumes of homogenising buffer and the homogenate was filtered through two layers of wet gauze and filtrate was centrifuged at 500g for 10min. The supernatant was subsequently centrifuged at 40,000g for 20 min. The pellet thus obtained was resuspended in same volume of assay buffer (HEPES 20 mM, EDTA 5mM, pH 7.4) and were stored at -70°C until the time of assay.

Ligand binding assay: The compounds were dissolved and diluted in DMSO. The membrane homogenates (150-250 µg protein) were incubated in 250 µl of assay buffer (HEPES 20 mM, pH 7.4) at 24-25°C for 3h. Non-specific binding was determined in the presence of 1 µM atropine. The incubation was terminated by vacuum filtration over GF/B fiber filters (Wallac). The filters were then washed with ice cold 50mM Tris HCl buffer (pH 7.4). The filter mats were dried and bound radioactivity retained on filters was counted. The IC_{50} and K_d were estimated by using the non-linear curve fitting program using G Pad Prism software. The value of inhibition constant K_i was calculated from competitive binding studies by using Cheng & Prusoff equation (*Biochem Pharmacol*, 1973; 22:3099-3108), $K_i = IC_{50} /(1+L/K_d)$, where L is the concentration of [^{3}H]NMS used in the particular experiment. $pK_i = -[\log K_i]$.

The Ki results of the compounds observed were in the range of 0.05 nM to 136 nM for M_3 receptor and 0.06 nM to 34.6 nM for M_2 receptor.

Functional Experiments using isolated rat bladder:

Methodology: Animals are euthanized by overdose of urethane and the whole bladder is isolated and removed rapidly and placed in ice cold Tyrode buffer with the following composition (mMol/L) NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 0.1; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.55 and continuously gassed with 95% O_2 and 5 % CO_2.

The bladder is cut into longitudinal strips (3mm wide and 5-6 mm long) and mounted in 10 ml organ baths at 30° C, with one end connected to the base of the tissue holder and the other end connected to a polygraph through a force displacement transducer. Each tissue is maintained at a constant basal tension of 2 g and allowed to

equilibrate for 1 hour during which the PSS is changed every 15 min. At the end of the equilibration period, the stabilization of the tissue contractile response is assessed with 1μmol/L of Carbachol consecutively, 2-3 times. Subsequently a cumulative concentration response curve to carbachol (10^{-9} mol/L to 3 X 10^{-5} mol/L) is obtained. After several washes, once the baseline is achieved, cumulative concentration response curve is obtained in presence of NCE (NCE added 20 min. prior to the second CRC).

The contractile results are expressed as % of control E max. ED_{50} values are calculated by fitting a non-linear regression curve (Graph Pad Prism). The pKB values are calculated by the formula pKB = - log [(molar concentration of antagonist/ (dose ratio-1))] where, dose ratio = ED_{50} in the presence of antagonist/ED_{50} in the absence of antagonist.

***In vivo* experiments using anesthetized rabbit:** The effect of test substances was studied on carbachol evoked changes on bladder pressure, heart rate and salivation.

Male rabbits weighing 1.2-3 kg were anaesthetized with urethane (1.5g/kg), and administered as a slow intravenous infusion through the marginal ear vein. The tracheae were cannulated to maintain airway patency. Blood pressure was recorded from the femoral artery by means of a Statham P10 EZ pressure transducer connected to a Grass model 7D polygraph. The heart rate was monitored by a tachograph triggered by the pulse wave of blood pressure. The other femoral artery was cannulated for the administration of carbachol. Test compound and saline were infused intravenously via the femoral vein.

The bladder was exposed through a midline laparotomy and both the ureters were identified, carefully separated and ligated. The ureters were incised proximally to allow free flow of urine from the kidney to the exterior. Bladder neck was gently held and the urethra was traced and separated from the adjoining tissues. PE canula was introduced into the bladder and ligated. The bladder was drained and subsequently filled with 15ml of warm saline (37°C). The other end of the intravesical catheter was connected to the Grass model 7D polygraph through a Statham P10 EZ pressure transducer to monitor the bladder pressure. Care was taken to keep the exposed area moist and warm. A period of 30-60 min was allowed for stabilization of parameters subsequent to surgery. Salivation response was assessed by placing preweighed absorbent cotton gauze in the buccal cavity for 2 minutes after carbachol administration.

The effect of the compound on carbachol (1.5μg/kg, intrarterial) induced changes on blood pressure, heart rate and bladder pressure were observed. At least two stable responses were obtained. These responses were considered as 100%. Subsequently, effect of increasing dose of test compound or vehicle (i.v,12 to 15 min before carbachol challenge) was studied.

The change in bladder pressure, salivation and agonist induced bradycardia were expressed as % change from pretreatment control. ID_{50} values (dose required to inhibit 50% of response) were calculated from non-linear curve fitting for sigmoidal dose response curve using Graph Pad Prism software and values were expressed as μg/kg. The ID_{50} values for bladder pressure for compounds tested ranged from about 1.89 to about 4.2 μg/kg. The ID_{50} values for salivation for compounds tested ranged from about 3.7 to about 30.4 μg/kg.

While the present invention has been described in terms of its specific embodiments, certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.